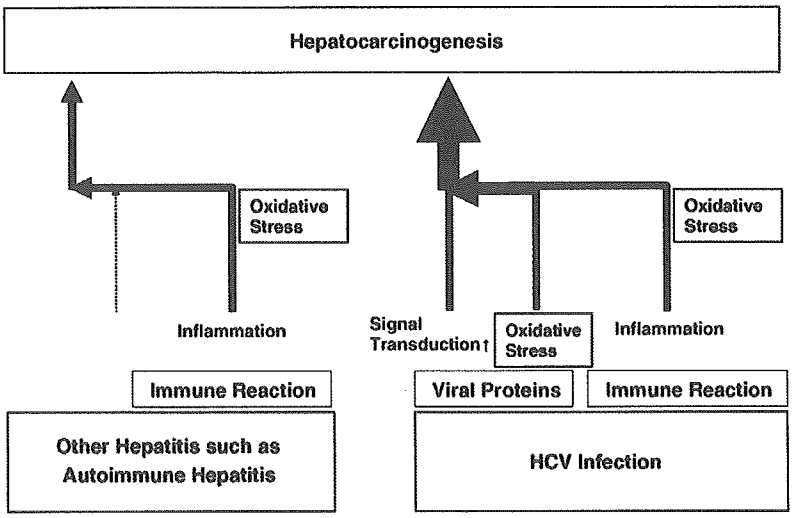

Fig. 5. Oxidative stress and hepatocarcinogenesis in various types of hepatitis (hypothesis). Oxidative stress is generated in all types of hepatitis via inflammation accompanied by continual cell death and regeneration. In HCV infection, HCV itself causes the production of oxidative stress in a synergy with inflammation. In this sense, the quality of "inflammation" in HCV infection may be different from that in other types of hepatitis. Additional impact of HCV proteins on the intracellular signal transduction would provoke the development of HCC. These may explain the conspicuous properties of HCC development.

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ORIGINAL ARTICLE

Is blood eosinophilia an effective predictor of acute rejection in living donor liver transplantation?*

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acute cellular rejection, eosinophilia, liver transplantation, steroid.

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Summary

The association of blood eosinophilia with acute cellular rejection (ACR) after living donor liver transplantation has not been examined yet. The subjects were the 167 recipients who underwent liver biopsy (314 times). The blood eosinophil counts in the preoperative period ($n = 167$), 3 days before ($n = 314$) and on the day of biopsy ($n = 314$) were compared among the groups stratified by severity of ACR. Among 314 biopsy specimens, the 140 biopsy specimens were diagnosed with ACR. In the 140 ACR episodes, eosinophil counts before and after therapy was compared between the episodes that responded to therapy ($n = 80$) and those not ($n = 60$). The sensitivity and specificity of preoperative eosinophilia (eosinophil counts >130 mm^3) to predict ACR was 33% and 65%, respectively. The eosinophil counts >400 mm^3 3 days before and on the day of biopsy was associated with the severity of ACR ($P < 0.0001$). The sensitivity to predict ACR was 26% and 33%, and the specificity, 94% and 93%, respectively. There was no significant difference in changes of eosinophil counts between the steroid-responders versus the nonresponders. The present results suggested the limited role of eosinophilia as a predictor of ACR after living donor liver transplantation.

Introduction

In liver transplantation, acute cellular rejection (ACR) is still a major complication that can lead to mortality. Early diagnosis is necessary for prompt treatment, which must be based on liver biopsy. Several reports indicate a relationship between blood eosinophilia and acute rejection in liver transplantation [1–4]. Infiltration of eosinophils into the graft and peripheral blood eosinophilia might relate to ACR. In most studies, eosinophilia preceded ACR by 2–4 days [1,5]. One report demonstrated a close relationship between pretransplantation peripheral blood eosinophilia and postoperative ACR [6]. All of these reports, however, were based on data from deceased donor liver transplantation. In living donor liver transplantation (LDLT), the relation between eosinophilia and ACR has not been examined.

It is controversial that whether there is a difference in the frequency of ACR rejection between LDLT and deceased donor liver transplantation [7,8]. Some authors

reported lower incidence of steroid resistant [9] or late onset ACR [10] after LDLT. This might be due in part to the length of graft cold ischemic time [7] or the HLA haplotype matching in living-related donor cases [9]. The difference in the frequency and severity of ACR between deceased donor liver transplantation and LDLT led us to examine whether blood eosinophilia can predict ACR after LDLT.

Patients and methods

Patients

Subjects were 305 consecutive patients that underwent LDLT at our hospital. Two patients complicated by chronic rejection and eight patients who underwent emergent transplantation were excluded. Of the remaining 299 patients, biopsies were performed in 167 patients consisting 131 adults [47 ± 1.0 (mean ± SE) in age] and 36 children (6.3 ± 1.0 years old). The indications for LDLT included HCV related cirrhosis ($n = 39$), hepatitis B virus related cirrhosis ($n = 14$), cirrhosis of other etiologies ($n = 7$), biliary atresia ($n = 37$), primary biliary cirrhosis ($n = 33$), primary sclerosing cholangitis ($n = 4$), autoimmune hepatitis ($n = 5$), fulminant hepatic failure ($n = 15$), metabolic diseases ($n = 7$) and others ($n = 6$).

Acute cellular rejection was diagnosed based on biopsy and graded into four classes according to the Banff scheme [11] [Grade 0 (G0): no evidence of rejection, Grade 1 (G1): mild rejection, Grade 2 (G2): moderate rejection, Grade 3 (G3): severe rejection; Fig. 1]. Postoperative immunosuppression was achieved with tacrolimus and methylprednisolone [12]. Tacrolimus was administered to control the trough level at approximately 16–18 ng/ml for the first week, and gradually tapered to 5–8 ng/ml over 6 months. Steroids were also tapered day by day from 3 mg/kg on the first postoperative day to 0.3 mg/kg on the fifteenth postoperative day. The dose was then decreased slowly to 0.06 mg/kg over 6 months. When the diagnosis of ACR was confirmed, 20 mg/kg of methylprednisolone was administered, which was then tapered by reducing the dose by half each day until the same dose as before therapy was achieved.

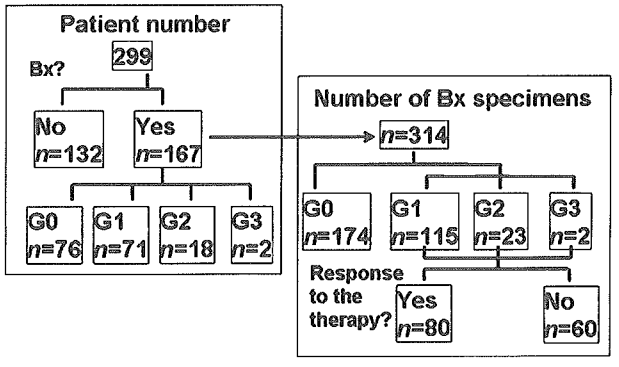

Figure 1 The numbers of the patients and liver specimens studied. Bx, liver biopsy.

Biopsy was performed when levels of all blood liver function tests, including transaminases, bilirubin, gamma-glutamyl transpeptidase and alkaline phosphatase, elevated. No protocol biopsy was performed.

Analysis

The relationship between preoperative eosinophilia and ACR stratified by grade was examined. Preoperative eosinophilia was defined as absolute eosinophil count (AEC) >130 mm^3 [6]. The relationship of eosinophilia 3 days before or on the day of biopsy and ACR grouped by grade was examined. Here, the number of eosinophils was evaluated as AEC or relative eosinophil count (REC: AEC × 100/total leukocyte count). Postoperative eosinophilia was defined as AEC more than 400/mm^3 and/or REC more than 4% [3].

Pre- or post-treatment AEC, REC, and eosinophil count changes were compared between patients that responded to the treatment and those that did not. Treatment was judged successful when transaminase and bilirubin levels improved to normal levels and did not increase again during the following month. If liver dysfunction recurred again within 1 month, followed by biopsy-proven ACR, the treatment was defined as failed.

Statistics

Data were expressed as mean ± SE. Sensitivity and specificity of eosinophilia was calculated for the prediction of ACR or improvement of ACR. AEC and REC were compared between groups using an unpaired *t*-test or one-way ANOVA. A *P*-value of <0.05 was considered statistically significant.

Results

Preoperative eosinophilia

An average of 2.2 biopsies were performed per patient. The interval between transplantation and biopsy was on 32 ± 2.0 days. The degree of ACR included G1 in 71, G2 in 18 and G3 in two patients. Other 76 patients showed only indeterminate evidence of ACR in every biopsy samples and were classified to G0. Preoperative AEC of the patients with and without postoperative ACR was 168 ± 27/mm^3 and 114 ± 16/mm^3, respectively ($P = 0.78$). There was no significant difference in REC (G0,

2.6 ± 0.34%; G1, 2.9 ± 0.52%; G2, 3.7 ± 0.98%; $P = 0.54$) or AEC (G0, 114 ± 18/mm^3; G1, 159 ± 27/mm^3; G2, 217 ± 51/mm^3; $P = 0.10$) among the G0–G2 grades of ACR (Fig. 2a). Two G3 specimens were excluded from the analysis. Preoperative eosinophilia predicted ACR with a sensitivity of 33% and a specificity of 65%, respectively (Table 1).

Eosinophilia 3 days before the biopsy

Eosinophil counts 3 days before the biopsy were available for 314 biopsy samples (Fig. 1), graded as G1 ($n = 115$) and G2 ($n = 25$). The other 174 samples showed indeterminate evidence of ACR and were classified to G0. The major findings the samples included nonspecific hepatitis with or without cholestasis ($n = 122$), congestion ($n = 15$), recurrent hepatitis C ($n = 15$) only mild lymphocyte infiltration or endothelialitis ($n = 5$), cholangitis ($n = 3$) and no abnormal findings ($n = 14$). REC and AEC 3 days before biopsy in patients complicated with ACR were 2.5 ± 0.3% and 234 ± 33/mm^3, respectively. REC and AEC in patients without ACR were 0.8 ± 0.1% and 77 ± 12/mm^3, respectively. When the biopsy samples were grouped according to the severity of ACR, there was a significant difference between the groups both in REC ($P < 0.0001$) and AEC ($P < 0.0001$; Fig. 2b). Eosinophilia (REC > 4%) 3 days before the biopsy predicted ACR with a sensitivity of 26% and a specificity of 94%, respectively (Table 1).

Eosinophilia on the day of biopsy

Eosinophil counts on the day of the biopsy were available for 314 biopsy samples. The REC and AEC on the day of the biopsy with findings of ACR were 3.3 ± 0.3% and 312 ± 35/mm^3, respectively, being significantly higher than those without ACR ($n = 174$, 0.8 ± 0.1%, $P < 0.0001$ and 78 ± 13/mm^3, $P < 0.0001$). When biopsy episodes were grouped according to the severity of ACR, there was a significant difference between groups both in REC ($P < 0.0001$) and AEC ($P < 0.0001$; Fig. 2c). Eosinophilia (REC > 4%) on the day of biopsy predicted ACR with a sensitivity of 33% and a specificity of 93%, respectively (Table 1).

Eosinophil count in response to treatment

Eosinophil count changes (count 1 week after treatment minus that just before treatment) could be calculated in the 140 biopsy episodes. Of these, 80 were responsive to steroid recycling therapy and 60 were resistant. Pretreatment REC and AEC were 2.8 ± 0.4% and 226 ± 35/mm^3 in the responding group and 4.0 ± 0% and 426 ± 65/mm^3 in the nonresponding group, respectively. Post-treatment REC and AEC were 2.3 ± 0.5% and 176 ± 32/mm^3 in the responding group and 2.6 ± 0.6% and 202 ± 55/mm^3 in the nonresponding group, respectively. There was a significant difference between groups in the pretreatment AEC ($P = 0.04$), but not in pretreatment REC ($P = 0.07$), post-treatment REC ($P = 0.49$), or post-treatment AEC ($P = 0.48$).

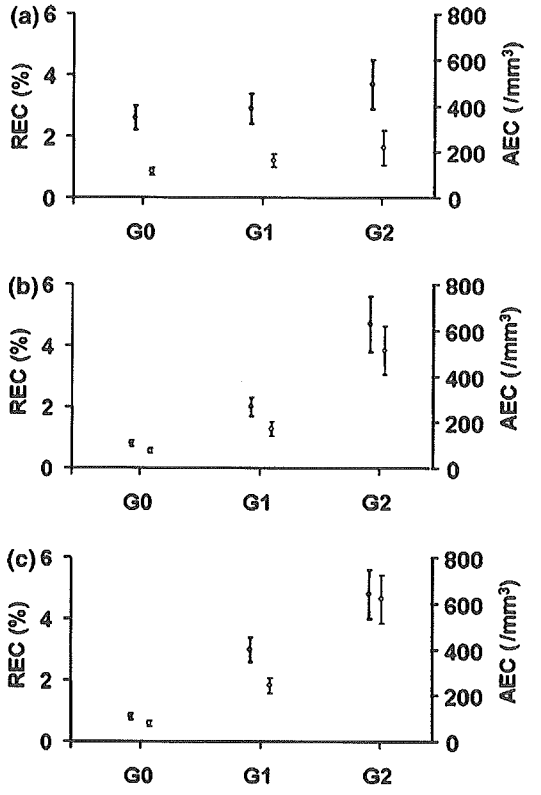

Figure 2 Relative (REC, thick bar and closed circle) and absolute eosinophil counts (AEC, thin bar and open circle) stratified by grade of rejection at preoperative (a) $n = 197$; 3 days before the biopsy (b) $n = 314$; and on the day of biopsy (c) $n = 314$. $P < 0.0001$ after comparison among the groups in the analyses of (b) and (c).

Table 1. Significance of eosinophil counts to predict acute cellular rejection.

Conditions	Events	Results	Sensitivity (%)	Specificity (%)
Pre-Tx	AEC > 130	ACR	33	65
Before Bx	REC > 4	ACR	26	94
	AEC > 400		20	95
On Bx	REC > 4	ACR	33	93
	AEC > 400		28	97
Before and after SRT	Decreased REC	Improvement of ACR	45	50
	Decreased AEC		50	43

Tx, transplantation; Bx, biopsy; SRT, steroid recycle therapy; ACR, acute cellular rejection; AEC, absolute eosinophil count; REC, relative eosinophil count.

Relative eosinophil count decreased in 36 and 30 treatments in the responding and nonresponding groups, respectively, whereas AEC decreased in 40 and 34 treatments. A decrease in REC or AEC predicted successful treatment of ACR with a sensitivity of 45% or 50% and a specificity of 50% or 43% (Table 1).

Discussion

Few studies have evaluated whether preoperative eosinophilia predicts ACR [6]. Nagral *et al.* [2] reviewed 129 biopsy cases. They demonstrated that there was no association between preoperative eosinophil count and the severity of ACR. They also demonstrated that AEC 1 or 2 days before or on the day of biopsy predicted ACR with low sensitivity (30.3–37.5%) and high specificity (83.3–91.8%). In our study also, eosinophilia both 3 days before and on the day of biopsy predicted ACR with low sensitivity and high specificity.

In contrast, Hughes *et al.* [13] emphasized that monitoring blood eosinophil count and serum eosinophil cationic protein was useful for early ACR diagnosis because they increase 2–3 days earlier than serum transaminase or alkaline phosphatase levels. Foster *et al.* [14] reported high sensitivity and specificity of blood eosinophilia in predicting ACR when they combined elevated serum transaminase or alkaline phosphatase levels. The exact reason for the discrepancy remains unclear, but might be due to a different dose of methylprednisolone for basal immunosuppression in our protocol: 3.0 mg/kg on the first postoperative day versus 1.5 mg/kg in Foster's report. The baseline eosinophil numbers might be decreased because of higher doses of steroid [15].

Our results indicated a higher pretreatment AEC in the steroid nonresponding ($426 \pm 65/mm^3$) compared with that of the responding group ($226 \pm 35/mm^3$, $P = 0.04$). They may support the phenomenon that the eosinophil count before or on the day of biopsy correlated well with the grade of ACR. A similar association was also reported by Barnes *et al.* [3] in liver transplantation and Trull *et al.* [15] in cardiac and lung transplantation. However REC was not a predictor of the response to the steroids, indicating the association between eosinophil counts before the treatment and the response to the treatment was not to be firm. Additionally the decrease in REC and AEC was not useful for predicting the effect of steroids on ACR in our series. Our results revealed a significant decrease in REC and AEC after steroid recycle therapy irrespective of the response to therapy. The finding might be explained by the hypothesis that steroids downregulate eosinophilia [16].

In summary, eosinophilia in the preoperative period, 3 days before and on the day of biopsy, predicted consequent ACR with high specificity, but low sensitivity. The present results suggested the limited role of eosinophilia as a predictor of ACR after LDLT.

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Living Donor Liver Transplantation for Patients With Hepatitis C Virus Cirrhosis: Tokyo Experience

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Living donor liver transplantation is an alternative therapeutic option for patients with end-stage HCV cirrhosis because of the cadaveric organ shortage. Preliminary results, however, indicate that live donor grafts might be disadvantageous for HCV patients. Sixty-seven patients underwent living donor liver transplantation for HCV cirrhosis between 1996 and 2004. All the patients preemptively received antiviral therapy consisting of interferon alfa-2b and ribavirin, which was started approximately 1 month after the operation. The therapy continued for 12 months after the first negative HCV RNA test. The patients were then observed without the therapy for 6 months. The therapy was continued for at least 12 months, even when the HCV RNA test remained positive. The subjects were removed from the protocol if they could not continue the therapy for 12 months because of adverse effects or could not start the therapy because of early death. Twelve patients were removed from the protocol as a result of early death (n = 9) or cessation of the drug (n = 3). Another 16 patients are currently on the protocol. Of the remaining 39 patients, 16 patients (41%) had a sustained virologic response. The cumulative 5-year survival of the HCV-positive patients was 84%, which was comparable with that of patients negative for HCV (n = 168, 86%). The present preemptive antiviral protocol after living donor liver transplantation is safe and warrants a controlled study to confirm its benefit on graft survival.

Living donor liver transplantation (LDLT) is now a common alternative procedure to deceased donor liver transplantation (DDLT), which reduces waiting-time mortality in an era of deceased donor shortage. By June 2003, 1275 LDLT cases were recorded in the European Liver Transplantation Registry.[1] The 3-year graft survival rates were 71%, although the survival rates of HCV-positive patients are unknown. In the United States,[2] 1526 adult LDLT cases were performed by May 2004. HCV is the most common indication for LDLT, and the number of HCV-positive patients is stable, approximately 100 per year between 2000 and 2002. According to the Japanese Liver Transplantation Society,[3] 1335 adult LDLT procedures were performed in Japan by the end of 2003, and of these 297 (22%) were performed for HCV cirrhosis.

A current debate in the field of liver transplantation is the possibility of increased severity of recurrent HCV infection in LDLT patients. If HCV recurs earlier and more severely after LDLT, a specific strategy for preventing the detrimental effects of HCV on living donor grafts must be developed. Preemptive interferon therapy (prophylaxis) during the early post-transplantation period might reduce the incidence and severity of HCV recurrence. In the present study, we report our results of LDLT for chronic hepatitis C and discuss the feasibility of an antiviral protocol.

Patients and Methods

We performed preemptive therapy for LDLT patients with HCV infection. From 1996–2004, 67 patients underwent LDLT for HCV cirrhosis at the Tokyo University Hospital. The patients were 51 men and 16 women, and their ages ranged from 23–63 years (median, 55 years). The HCV genotype was 1b in 53 patients (79%). Forty-one patients (61%) had hepatocellular carcinoma. Our surgical technique for recipient and donor surgery is described elsewhere.[4] All the patients received the same immunosuppressive regimens with tacrolimus (Prograf; Astellas Pharma Inc, Tokyo, Japan) and methylprednisolone as described previously.[5]

All the patients preemptively received antiviral therapy consisting of interferon alfa-2b and ribavirin, which was started approximately 1 month after the operation. The therapy was continued for 12 months after the first negative HCV RNA test. The standard regimen included interferon alfa-2b (3 million units [MU] ×3 per week) and ribavirin (800 mg/day) for 6 months. The patients were then observed without the therapy for 6 months. The therapy was continued for at least 12 months, even if the HCV RNA test remained positive.

Therapy was discontinued when there was significant leukopenia (<1500/mL), thrombocytopenia (<50,000/mL) de-

Abbreviations used in this paper: DDLT, deceased donor liver transplantation; LDLT, living donor liver transplantation.

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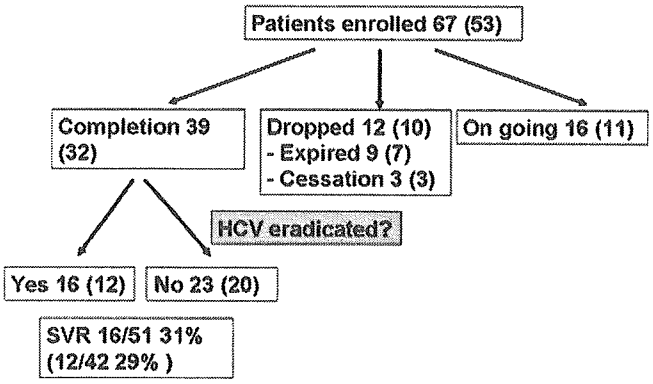

Figure 1. Results after preemptive antiviral therapy in University of Tokyo Hospital. *Numbers in parentheses* indicate those of genotype 1b. *SVR*, sustained viral response ratio. HCV eradicated? = Was the patient negative for HCV (<1000 copies/mL)?

spite application of granulocyte colony-stimulating factor (Gran; Sankyo Co Ltd, Tokyo, Japan), hemolytic anemia (hemoglobin <8 g/L), renal dysfunction (serum creatinine >2 mg/dL), depressive psychologic status, or general fatigue. The subjects were removed from the protocol if they could not continue the therapy for 12 months because of adverse effects or could not start the therapy as a result of early death.

Blood counts and liver function test results were checked every 2 weeks for the first month and at 4-week intervals thereafter. Serum samples were collected once a month for quantitative HCV RNA detection. Protocol liver biopsy was not performed. The log-rank test was used to compare the survival rate of the HCV-positive patients with the HCV-negative patients who underwent transplantation during the same period (n = 168).

Results

A total of 28 patients were excluded from the analysis (Figure 1). Twelve patients were removed from the protocol because of early death (n = 9) or because of drug cessation (n = 3). Another 16 patients are currently on the protocol and were therefore excluded from the analysis. Of the remaining 39 patients, 16 (41%) obtained a sustained virologic response. The cumulative 5-year survival of the HCV-positive patients was 84%, comparable with that of patients negative for HCV (n = 168, 86%).

Discussion

Because interferon is more effective in patients with a lower viral load,[6] initiating preemptive therapy before peak viral loads are reached is a rational approach. There is, however, a theoretical risk of increasing cellular rejection, as observed in kidney and liver transplantation.[7] Preemptive therapy during the early post-transplantation period with interferon in combination with ribavirin has been attempted in DDLT.

In a case series by Mazzaferro et al,[8] 36 recipients were treated with interferon alfa-2b (3 MU ×3 per week) and ribavirin (10 mg · kg^{-1} · day^{-1}). Treatment was started a median of 18 days after the operation and continued for 11 months. After a median follow-up of 52 months, the 5-year patient survival was 88%. Serum HCV RNA clearance was obtained in 12 patients (33%). No further antiviral treatment was required because of negative HCV RNA in serum and normal liver histology for a median of an additional 36 months. In another study,[9] 63 patients (<50% of screened cases) were randomized within 4 weeks after DDLT and treated for 48 weeks: 20 control subjects, 21 interferon alone, and 22 interferon and ribavirin. At 2 years, HCV RNA was negative in 13%, 13%, and 33%, respectively. Remarkably, there was no histologic recurrence in patients with a sustained viral response.

The association between LDLT and early HCV recurrence remains to be determined,[10] although most of the recent reports suggest that living donor graft has no effect on short-term outcome or severity of virus recurrence. Reports from New York-Presbyterian Hospital[11] indicate that the time to diagnosis of recurrent HCV is significantly shorter in LDLT. Other data indicate that the 5-year survival of HCV patients (n = 69) who undergo LDLT is 64%, which is comparable with that of DDLT patients (n = 202, 69%). The multicenter adult to adult LDLT cohort study (A2ALL) might soon provide answers to questions about recurrent HCV after LDLT and DDLT.[12]

In areas where the cavaderic organ source is almost negligible, LDLT must be selected as a therapeutic option, regardless of any potential additional risk. The results of LDLT for HCV cirrhosis in our hospital were comparable with those for non-HCV patients. If living donor graft is associated with early HCV recurrence and consequently poorer graft survival, an aggressive antiviral protocol should be performed to improve the outcome of LDLT for HCV. The present data indicate that the protocol after LDLT is safe and warrants a controlled study to confirm its benefit for graft survival.

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Clinical Transplantation

Splenectomy and preemptive interferon therapy for hepatitis C patients after living-donor liver transplantation

Kishi Y, Sugawara Y, Akamatsu N, Kaneko J, Tamura S, Kokudo N, Makuuchi M. Splenectomy and preemptive interferon therapy for hepatitis C patients after living-donor liver transplantation.
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Abstract: Recurrent hepatitis C after liver transplantation is a major cause of graft failure. We routinely perform preemptive interferon and ribavirin therapy in patients after living-donor liver transplantation indicated for hepatitis C-related cirrhosis. One of the obstacles for the therapy includes blood cytopenia. To overcome this problem, we recently performed splenectomy concurrently with liver transplantation. Thirty-five patients underwent liver transplantation and received preemptive therapy for hepatitis C. They were divided into two groups: those with splenectomy (group A, n = 21) and those without (group B, n = 14). There was no significant difference in the frequency of morbidity between the groups. Platelet counts were well maintained in group A patients during the therapy, and cytopenia led to the discontinuation of the therapy in one group B patient. The results of the preliminary study warrant a randomized control trial to examine the feasibility of splenectomy and preemptive viral therapy during liver transplantation for hepatitis C.

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Key words: hepatitis C – interferon – liver transplantation – splenectomy – thrombocytopenia

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Hepatitis C virus (HCV) infection is one of the leading etiologies for liver transplantation. The main problem of the post-transplantation course is recurrent hepatitis with 11–14% of recipients redeveloping hepatitis leading to graft failure (1, 2). However, retransplantation provides poor results, with a 3-yr survival rate of only 40–56% (3, 4).

Although interferon (IFN) and ribavirin therapy is one of the standard treatments, the sustained virologic response ratio of the therapy for recurrent HCV after transplantation is limited to approximately 30% (5–7). We routinely perform preemptive IFN therapy for recipients of living-donor liver transplantation (LDLT) indicated for HCV cirrhosis (8). One of the obstacles for starting or continuing combined IFN and ribavirin therapy includes blood cytopenia. To overcome this problem, we recently performed splenectomy concurrently with liver transplantation (9). Here we analyze the results of these patients to evaluate the feasibility of simultaneous splenectomy and combined therapy against HCV.

Patients and methods

From January 1996 to September 2004, 165 adult patients underwent LDLT. Of these, 39 recipients were indicated for HCV cirrhosis and received preemptive IFN and ribavirin therapy. Of these, four were excluded from the study because two died before the start of therapy due to uncontrolled cytomegalovirus infection or resistant acute cellular rejection, and two patients were followed up at

other hospitals and detailed laboratory data could not be obtained. The remaining 35 patients were the subjects of this study. They were divided into two groups: those with splenectomy (group A, n = 21) and those without (group B, n = 14).

The protocol of the preemptive IFN and ribavirin therapy was reported previously (8). In brief, the therapy was started when the white blood cell count was >4000 mm^3, hemoglobin level >10 g/dL, and platelet count $>100\,000/mm^3$. The therapy was initiated with 3 million units of IFN-alpha2b (Intron A; Schering-Plough K.K., Osaka, Japan) three times per week and 400 mg of ribavirin per day, which was increased up to twice the initial dose according to patient tolerance. The therapy was discontinued when there was significant leucopenia ($<1500/mm^3$), thrompocytopenia ($<50\,000/mm^3$) despite application of granulocyte colony-stimulating factor (G-CSF), hemolytic anemia (hemoglobin level <8 g/dL), renal dysfunction (serum creatinine >2 mg/dL) or depressive psychologic status.

Preoperative blood cell count, platelet count (mm^3), leukocyte count (mm^3), and hemoglobin (g/dL) were taken just before IFN therapy, and the numbers of days from transplantation to the start of therapy were evaluated. Blood cell counts during the therapy were examined weekly for the first month, monthly for the first year, and annually later on. The frequency of discontinuation of the therapy and its cause were reviewed. Completion of the therapy was defined as the elimination of HCV (<500 copies/mL by Amplicor HCV; Roche Molecular Systems, Pleasanton, CA, USA). Here, HCV was considered to be eliminated when the serum HCV-RNA level was consistently negative for at least 6 months after cessation of combination therapy. Protocol liver biopsy was not performed.

Data are expressed as median and range. Statistical comparison was performed using Mann–Whitney test, Fisher's exact test or repeated measure analysis of variance where appropriate. p-value <0.05 was considered statistically significant.

Results

Patient profiles

In the 17 patients of group A, the duration between LDLT and starting the therapy ranged from 18 to 59 d (Table 1). In the other four patients of group A, it was longer than 2 months as we had to wait till they recovered from pneumonia, abdominal abscess, heart failure or renal failure. The number of the patients of HCV genotype 1b (HCV_{1b}) and those of the other genotypes (HCV_{non1b}) was 5 of 16 in group A and 2 of 12 in group B. There was no significant difference in preoperative blood cell counts or liver function between the groups.

Table 1. Patients profiles

Group	A (n = 21) Median	A (n = 21) Range	B (n = 14) Median	B (n = 14) Range	p-value
MELD score	14	4–34	10.9	2.4–25.3	0.22
Preoperative plt ($\times10^4/mm^3$)	5.0	2.9–13.5	5.6	4.1–15.0	0.30
Preoperative WBC ($\times10^3/mm^3$)	3.3	1.3–20.5	2.8	1.6–9.8	0.51
Preoperative Hb (g/dL)	9.0	5.5–12.7	10.5	5.6–13.3	0.24
Start day (d)	41	18–120	30	7–130	0.34
HCV-RNA before therapy (kcopies/mL)	663	186–3350	510	46–1700	0.66

MELD, model for end-stage liver disease; plt, platelet; WBC, white blood cell; Hb, hemoglobin.

Postoperative infectious diseases

In group A, six (29%) patients suffered from infectious disease: four from abdominal abscess, one from fungal pneumonia and one from bacterial pneumonia. Two of the four abdominal abscesses were related to the splenectomy because there was pancreatic juice leakage from the drainage tube in the left subphrenic space. Both of the patients responded well to surgical re-exploration. In group B, five (36%) patients had infection episode with no mortality including three abdominal abscesses, one sepsis and one osteomyelitis.

Blood cell counts after interferon and ribavirin therapy

In group A patients, platelet count significantly increased soon after LDLT and was maintained during the treatment for up to 2 yr (Fig. 1). Platelet count was kept higher in group A patients ($p = 0.008$) during the observation period. Leukocytopenia $<3000/mm^3$ were observed in three patients of group A and seven in group B. All of them were well controlled by G-CSF except for one in group B who discontinued the therapy because of cytopenia.

Continuation of therapy

Six (29%) patients in group A and three (21%) in group B discontinued therapy before the HCV was eradicated (Table 2). A 40-yr-old male in group A underwent retransplantation for cholestatic

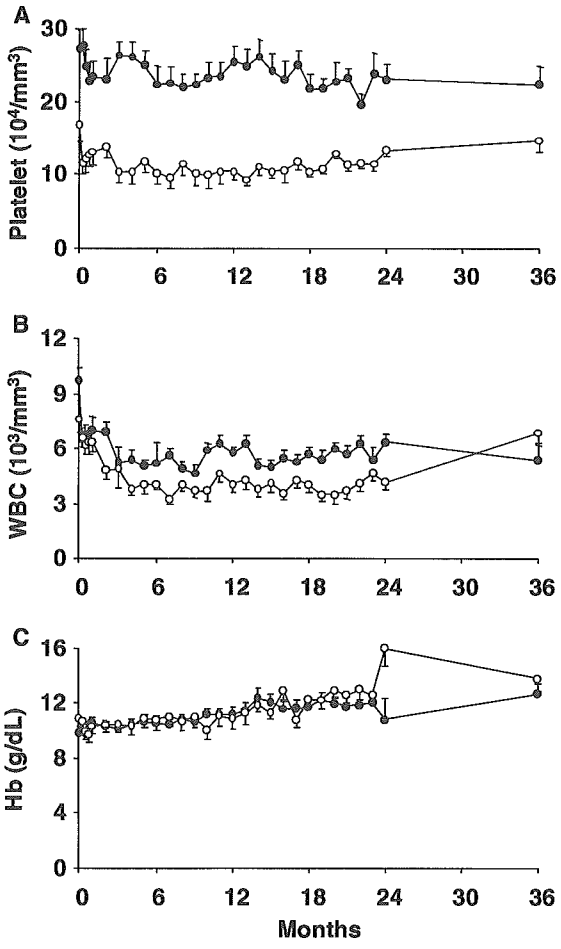

Fig. 1. Changes of platelet (A), white blood cell count (B) and hemoglobin (C). Levels after interferon therapy in group A (thick line with close circles) and group B (thin line with open circles). The bar represents a standard error value. There was a significant difference between the groups in the platelet count ($p = 0.008$).

Table 2. Timing [months after the start of interferon (IFN) therapy] and the reason of cessation of IFN therapy

Group	Patient	Timing	Reason
A	1	14	Renal dysfunction
	2	7	Depression
	3	7	Death caused by thrombotic thrombocytopenia
	4	18	Retransplantation because of cholestatic hepatitis
	5	19	Renal dysfunction
	6	3	Depression
B	1	4	Death caused by virus associated hemophagocytotic syndrome
	2	9	Thrombocytopenia
	3	6	Death because of hepatocellular carcinoma recurrence

hepatitis 18 months after the primary LDLT and died of liver failure 4 months after the retransplantation. Four patients in group A and three in group B completed the therapy. Eleven patients in group A and eight in group B continued the therapy for 21 (range: 11–47) and 24 (range: 11–66) months, respectively.

Effect of genotype

In group A, HCV-RNA became negative in 44% (7/16) of HCV_{1b} patients and 60% (3/5) of the HCV_{non1b}. Median periods of treatment until the RNA level became negative was 15 (range: 1–18) months and 2 (range: 2–8) months in each group, respectively. There was no significant difference in the period by genotype ($p = 0.30$). In group B, HCV-RNA became negative in 17% (2/12) of HCV_{1b}, and 100% (2/2) of HCV_{non1b}.

Discussion

Preemptive IFN and ribavirin therapy to prevent cholestatic hepatitis has not been established. Only a few centers, including ours, report using this strategy (8, 10–13). Among the 39 patients who underwent preemptive IFN therapy after liver transplantation with or without splenectomy, we experienced cholestatic hepatitis in only one patient, which might indicate the possibility that long-term IFN and ribavirin therapy prevents the occurrence of cholestatic hepatitis. Gopal and Rosen (14) reported the results of IFN and ribavirin therapy in seven cholestatic hepatitis patients with only two patients who survived for an average of 32 months. They emphasized the importance of continuing the therapy indefinitely because the cessation of the therapy even after 12 months or more of treatment with sustained HCV-RNA negativity led to rapid recurrence of cholestatic hepatitis. IFN and ribavirin therapy might be worth continuing over the long term, especially in patients with HCV_{1b}. The preemptive therapy is effective in cases with lower HCV-RNA levels and less graft injury by the virus (11, 13). Accordingly, the treatment should be started within a short interval of transplantation.

The indications for simultaneous splenectomy in liver transplantation for reducing portal hypertension to protect the graft from congestion, especially in small left liver graft, or repairing portal flow regurgitation are established (15, 16). The effectiveness of splenectomy against thrombocytopenia is reported (9, 17). Several authors, however, have objected to perform splenectomy as a therapeutic option for thrombocytopenia because it might increase the risk of septic complications postoperatively, and instead recommend splenic artery ligation or radiologic partial splenic embolization (18–21). Several reports, however, suggest that

the indication of such ligation or embolization methods should also be considered with care because of the low success rate and risk of complications (22, 23). We previously reported the safety of concomitant splenectomy and several other centers report similar good results (9, 24). The results of the present study suggest that splenectomy is feasible for starting combination therapy early after transplantation and continuing for up to 4 yr with an acceptable morbidity rate.

The long-term effect of splenectomy as a therapeutic option for blood cytopenia because of portal hypertension remains unclear in patients undergoing IFN and ribavirin therapy. Randomized control trials to examine the risk and benefits of splenectomy for patients undergoing liver transplantation and combined therapy for hepatitis C are necessary.

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Efficacy and Immunologic Responses to Influenza Vaccine in HIV-1–Infected Patients

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Summary: Influenza vaccine is recommended for HIV-1–infected patients. The present prospective study was conducted to evaluate the clinical efficacy and immunologic responses to the vaccine. From November 1 to December 27, 2002, 262 HIV-1–infected patients received a trivalent influenza subunit vaccine, whereas 66 did not. Influenza illness occurred in 16 vaccinated and 14 nonvaccinated patients (incidence = 6.1% [95% confidence interval (CI): 4%–10%] in vaccinated vs. 21.2% [CI: 13%–35%] in nonvaccinated persons, $P < 0.001$; relative risk = 0.29 [CI: 0.14–0.55]). Influenza vaccine provided clinically effective protection against influenza illness in HIV-1–infected patients. In baseline antibody-negative patients, anti-H1 and anti-H3 antibody responses to the vaccination were significant in those patients with a CD4 count >200 cells/μL compared with those with a CD4 count <200 cells/μL ($P < 0.05$). In contrast, in baseline antibody-positive patients, good antibody responses were observed irrespective of CD4 counts, like the healthy controls. Based on these results, annual vaccination is recommended. Specific CD4 responses correlated with HIV-1 viral load (VL), especially in patients treated with highly active antiretroviral therapy (HAART) compared with those without HAART ($P < 0.01$), although the clinical efficacy did not correlate with HIV-1 VL. HAART may enhance the immunologic efficacy of influenza vaccine.

Key Words: HIV-1, influenza, vaccination, antibody response, specific CD4

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After the recent approval of various anti-influenza drugs and rapid diagnosis kits for influenza infection by the Ministry of Health, Labor, and Welfare of Japan, it has become easier to diagnose this infection. Along with the developments in diagnostic methods and treatment of the infection, influenza vaccination programs have been actively applied in HIV-1–infected individuals. Influenza virus infection may be more prolonged in individuals with immunodeficiency[1] and can cause a transient increase in plasma HIV-1 viral load (VL)[2] that might become relevant to the clinical course of HIV-1 infection.[2,3] Therefore, influenza vaccine has been generally recommended for HIV-1–infected patients,[4–6] as is already stated in the guidelines of the Advisory Committee on Immunization Practices.[7] Few studies have reported the protective effect of such vaccination in patients with HIV-1 infection, however. Previous studies demonstrated that the number of CD4 T cells (CD4 count) could predict the efficacy of and/or antibody response to the vaccine but did not clearly demonstrate the correlation between the vaccine efficacy and HIV VL.[1,8–15]

Activated memory $CD4^+$ T cells are the predominant target of HIV-1,[16] and the antibody response to hemagglutinin (HA) is T-cell dependent.[17–19] Therefore, highly active antiretroviral therapy (HAART) may reconstitute the immune function of not only the antibody responses but T helper (Th)–cell responses. In this large prospective clinical study, we investigated the clinical efficacy of influenza vaccine in HIV-1–infected patients and correlated it with the immune response to the vaccine as determined by increased antibody titer and/or HA-specific CD4 T cells.

MATERIALS AND METHODS

Study Design and Participants

A 0.5-mL dose of single-shot trivalent influenza subunit vaccine, which contains 15 μg of influenza virus strains A/New Caledonia/20/99 (H1N1), A/Panama/2007/99 (H3N2), and B/Shanton/7/87, was prepared for adults in the 2002 through 2003 winter season in Japan. All HIV-1–infected patients who consulted the outpatient clinic of the AIDS Clinical Center at the International Medical Center of Japan from November 1 to December 27, 2002 were advised to receive the vaccine, although the final decision was left to the individual. In previous seasons, nearly half of HIV-1–infected patients received influenza vaccine in our clinic. This study was designed to be prospective in nature but nonrandomized. Only individuals, vaccinated and nonvaccinated, who understood the purpose of the study were enrolled, without any incentives. To keep selective bias to a minimum, all vaccinated and consecutive first-come 100 nonvaccinated patients were asked to participate in this study. All study participants gave

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informed consent, and the institutional ethical committee approved this study (protocol IMCJ-141). Twenty-six hospital staff members who were vaccinated with the same vaccine batch were enrolled as healthy immunized controls after consenting to participate in this study. Among them, 4 had no anti-influenza antibodies before vaccination. All participants were asked to visit to our clinic at least at week 0, 8, and/or 16 after enrollment to allow the withdrawal of 17 mL of blood at each visit for analysis of immunologic responses and routine examinations, including CD4 count and HIV VL.

Definition and Diagnosis of Influenza Virus Infection

In this study, influenza infection (illness) was defined if the patient had flulike symptoms associated with at least 1 adjunct diagnosis such as a serologic or virologic diagnosis. Flulike symptoms were defined as a fever of ≥38.0°C combined with 2 of the following 5 clinical symptoms: cough, rhinitis, myalgia, sore throat, and headache. All participants were asked to visit the clinic if they developed flulike symptoms. To avoid a bias in the clinical diagnosis, a history of influenza vaccination was written out on a separate colored sheet, which was removed from medical records before the outpatient clinic physician attended and examined the patient. The serologic diagnosis was defined as a >4-fold rise in anti-influenza antibody titer compared with before and 4 weeks after the symptoms. In addition, a change of the antibody titer from <10 to 40 U was defined as a 4-fold rise. Patients who had only the antibody rise but no flulike symptoms were not considered to have influenza-related illness. The virologic diagnosis was made by means of viral culture and/or a Rapidvue influenza test kit (Quidel, San Diego, CA) using a nasal or throat swab.

Laboratory Investigations

At each visit, CD4 T cells were enumerated by standard flow cytometry and HIV VL was measured using the Roche Amplicor assay kit, version 1.5 (Roche Diagnostic Systems, Branchburg, NJ). Antibody responses to each of the 3 individual vaccine components were examined by the standard hemagglutinin inhibition (HAI) assay.[20] Titers ≥40 U were defined as protective, and a >4-fold rise in the antibody titer was considered an adequate response in previously antibody-negative patients.

For assessment of HA-specific CD4 T-cell responses, intracellular γ-interferon (IFN) production was examined by flow cytometry using the method described previously.[21,22] Because of the limited availability of peripheral blood mononuclear cells (PBMCs), we analyzed the H1-specific CD4 T cells only. Because fresh PBMCs must be used for this assay, as a result of a labor limitation, only the first 10 participants per day were examined on any particular day. Briefly, HA was purified from influenza virus strain, A/New Caledonia/20/99 (H1N1), as described previously.[23] PBMCs were isolated from the fresh heparinized blood and cultured (2×10^6 cells/mL) with diluted H1 plus anti-CD28 antibody (1 μg/mL) or medium alone for 16 hours at 37°C. Brefeldin A (10 μg/mL) was added to each sample in the final 5 hours of incubation. After 16 hours of stimulation, the cells were collected and stained with anti-CD4 allophycocyanin antibody (Beckman Coulter, Fullerton, CA) and anti-CD69–fluorescent isothiocyanate antibody (Becton Dickinson). Subsequently, the cells were fixed and permeabilized to examine for the intracellular production of γ-IFN as described previously.[21,22] The flow cytometry analysis was performed by means of the FACSCalibur fluorescence-activated cell sorter with CellQuest software (BD Biosciences, San Jose, CA), and 10,000 CD4 T cells were collected for each analysis.

Statistical Analysis

The data on HA-specific CD4 T cells are presented as the arithmetic mean ± SEM. The data on anti-HA antibody titer are presented as the geometric mean. Statistical analyses were performed using StatView 5.0 software (Abacus Concepts, Berkeley, CA). Differences in the proportion of influenza virus infection between vaccinated and nonvaccinated groups were analyzed by the χ^2 test. Multiple logistic regression analysis was used to identify factors that contributed to protection against influenza illness. For the analyses of immune responses, participants were stratified by their CD4 count or HIV VL. Changes in antibody titer and HA-specific CD4 T cells were analyzed using the Kruskal-Wallis test or the Mann-Whitney U test. In all tests, a P value <0.05 was considered significant.

RESULTS

Subjects

During the period of vaccination, 626 HIV-1–infected patients visited our clinic, and 332 of these received the vaccine, whereas 294 did not. Among them, 317 of those vaccinated and 87 of 100 approached to participate as nonvaccinated patients agreed to participate in the present study. Consequently, 76 patients dropped out of the study (55 of 317 vaccinated patients and 21 of 87 nonvaccinated patients). There were no characteristic differences at baseline between the analyzed and drop-out patients (data not shown). None of the patients dropped out from the study because of HIV-1 disease progression, and none received anticancer or immunosuppressive agents during this study. The final composition of the study group based on compliance with the study protocol, including visits on the fixed dates, was 262 vaccinated (82.6%) and 66 nonvaccinated (75.9%) patients (Fig. 1). Table 1 summarizes the baseline characteristics of the participants.

Efficacy of Influenza Vaccine

The peak of the influenza epidemic of the 2002 through 2003 winter season in Japan was documented during the fourth week of January 2003 and was predominantly caused by influenza A/H3N2. The prevalence of influenza infection in this season was the third highest in the last decade.[24] In this study, 30 participants were diagnosed as having definitive influenza illness (5 patients with A/H1N1 strain, 16 with A/H3N2 strain, and 9 with B strain). Six patients were confirmed to have an influenza illness by flulike symptoms, positive viral cultures, positive influenza test kit results, and a >4-fold rise in antibody titer (1 with H1N1 strain, 1 with H3N2 strain, and

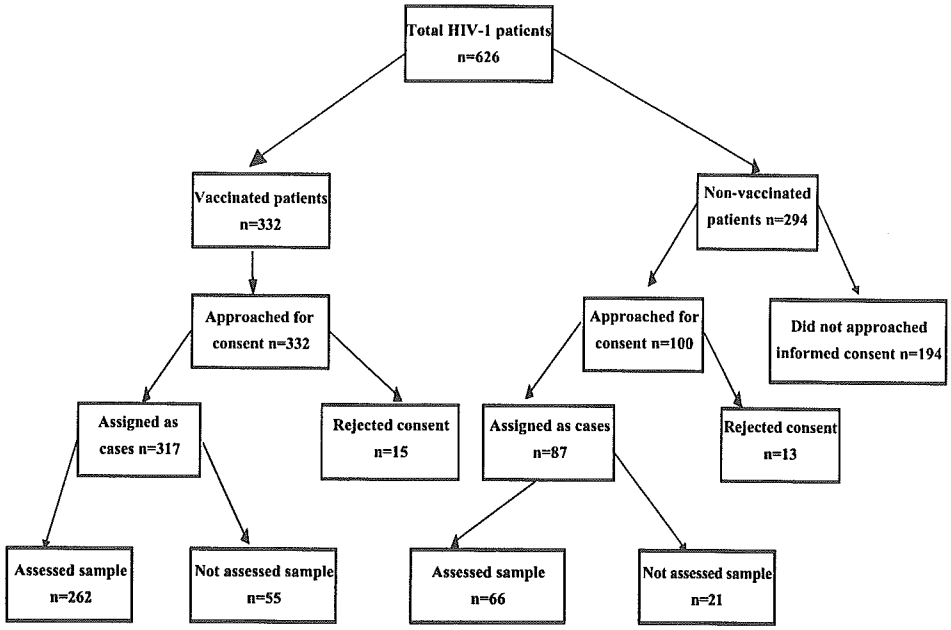

FIGURE 1. Profile of participants in this study.

4 with B strain); 3 by the symptoms, positive viral cultures, and antibody rise (2 with H1N1 strain and 1 with H3N2 strain); 5 by the symptoms, influenza test kit results, and antibody rise
(1 with H1N1 strain, 2 with H3N2 strain, and 2 with B strain); and 16 by the antibody rise between the symptoms (1 with H1N1 strain, 12 with H3N2 strain, and 3 with B strain). In total, 16 of 262 vaccinated patents had influenza illness (6.1%, confidence interval [CI]: 0.04–0.1) and 14 of 66 nonvaccinated patients had the illness (21.2%, CI: 0.13–0.35). The difference in the incidence between the 2 groups was significant ($P < 0.001$). The relative risk (RR) of influenza illness in vaccinated patients was 0.29 (CI: 0.14–0.55; $P < 0.001$) compared with nonvaccinated patients (Table 2). Eight patients who had a >4-fold rise in anti-H3 antibody titers between week 8 and week 16 without any clinical symptoms were not regarded as having influenza illness.

In patients with a CD4 count >200 cells/μL, the incidence of influenza illness in vaccinated patients (6.2%) was significantly lower than in nonvaccinated patients (21.0%) ($P < 0.001$). Conversely, in patients with a CD4 count <200 cells/μL, the same comparison showed no significant difference. Nevertheless, the incidences of influenza illness in vaccinated (5.9%) and nonvaccinated (22.2%) patients were the same as the incidence in patients with a CD4 count >200 cells/μL. Therefore, this analysis had lack of power because of the small number of nonvaccinated patients in this stratum. In vaccinated and nonvaccinated patients, the differences in the incidence were significant in patients with HAART ($P < 0.002$) and without HAART ($P < 0.05$) (see Table 2). When CD4 count was entered as a continuous variable, multivariate analysis using the logistic regression model identified vaccination ($P < 0.001$) and CD4 count ($P < 0.05$) but not HIV VL as independent predictors of influenza illness in HIV-1–infected patients.

In patients with influenza illness, 4 of 16 vaccinated patients and 4 of 14 nonvaccinated patients received an anti-influenza drug. None of the patients with influenza illness developed pneumonia that required treatment or hospitalization during the study period. Vaccination did not significantly change the HIV VL or CD4 count at weeks 8 and 16.

TABLE 1. Baseline Clinical and Immunologic Characteristics of Participants*

	Vaccinated	Nonvaccinated	*P*
No. participants (n)	262	66	—
Male/female ratio	7:1	15:1	n.s.
Median age, y (range)	41 (20–78)	40 (20–61)	n.s.
Received HAART (%)	75.2%	72.3%	n.s.
Median CD4 count at vaccination, μL (range)	380 (40–1137)	374 (66–1025)	n.s.
Median CD8 count at vaccination, μL (range)	778 (54–2649)	751 (163–1929)	n.s.
Median HIV VL at vaccination, $\log_{10}$/mL (range)	2.5 (1.5–6.2)	2.5 (1.5–6.4)	n.s.
Prior anti-H1 antibody-positive (%)	29.4%	26.4%	n.s.
Prior anti-H3 antibody-positive (%)	32.3%	30.3%	n.s.

*All participants were Japanese.
n.s. indicates not significant.

Anti-Hemagglutinin Antibody Responses Before and After Vaccination

HAI antibody titers against HA antigens (H1 and H3) were tested before and 8 and 16 weeks after vaccination (Table 3). To evaluate the effect of the single-shot influenza vaccine, subjects were divided into 2 groups based on the HAI titer before vaccination: the baseline HAI antibody-negative and antibody-positive groups. Furthermore, we excluded from this

TABLE 2. Incidence of Influenza Illness

	Vaccinated		Nonvaccinated		
	Illness/Patients	Rate (95% CI)	Illness/Patients	Rate (95% CI)	χ^2 Test
All patients	16/262	6.1% (0.04–0.1)	14/66	21.2% (0.13–0.35)	$P < 0.001$
CD4 count					
<200 cells/μL	3/51	5.9% (0.02–0.15)	2/9	22.2% (0.06–0.55)	n.s.
≥200 cells/μL	13/211	6.2% (0.03–0.1)	12/57	21.0% (0.12–0.33)	$P < 0.001$
HAART					
+	12/197	6.1% (0.04–0.1)	10/48	20.8% (0.11–0.34)	$P < 0.002$
–	4/65	6.2% (0.02–0.14)	4/18	22.2% (0.09–0.45)	$P < 0.05$

Incidence of influenza illness in healthy immunized controls was 3.8% (1 of 26, 95% CI: 0.01–0.19).
n.s. indicates not significant.

analysis the 13 patients who received the vaccination but had influenza illness (5 with H1N1 strain and 8 with H3N2 strain) during the study period so as to evaluate the antibody responses by the vaccination. The 8 patients who showed a >4-fold rise in anti-H3 antibody titers between week 8 and week 16 without any clinical symptoms were also excluded from this analysis, because the antibody rise in these cases was thought to be caused by influenza virus but not by vaccination. In the baseline HAI-negative group, the antibody responses to both antigens were significantly different compared with those in stratified HIV-1–infected patients by CD4 count (<200 cells/μL and ≥200 cells/μL; $P < 0.05$) at week 8 and week 16. These titers were low compared with those of the healthy immunized controls in both strata, however. In those with a CD4 count <200 cells/μL, 12 (27.9%) of 43 patients and 12 (32.4%) of 37 patients showed more than a 4-fold rise in the antibody responses against anti-H1 and anti-H3, respectively. In contrast, in those patients with a CD4 count >200 cells/μL, 62 (44.6%) of 139 patients and 61 (46.9%) of 130 patients showed a >4-fold rise in the antibody responses against anti-H1 and anti-H3, respectively. Although differences in the percentages of patients who showed both anti-H1 ($P = 0.05$) and anti-H3 ($P = 0.12$) antibody responses of the different CD4 strata were only marginal, there was a tendency for the single-shot vaccination to be more effective in terms of antibody responses in patients with a CD4 count >200 cells/μL. The antibody responses in both groups were not influenced by HIV VL (<100 copies/mL and ≥100 copies/mL; data not shown).

In the baseline HAI antibody-positive group, HAI titers to both antigens remained high and the sustainability of the antibody titers in HIV-1–infected patients was similar to those of the healthy controls, irrespective of CD4 counts (see Table 3). In terms of the antibody rise, in those with a CD4 count <200 cells/μL, 5 of 8 patients and 1 of 6 patients showed more than a 4-fold rise in the antibody response against anti-H1 and

TABLE 3. Anti-HA Antibody Responses After Vaccination in Baseline Anti-HA Antibody-Negative and Positive Individuals

	Anti-HA Antibody Responses* After Vaccination in HIV-1 Patients†						Healthy Immunized Controls	
	Stratum 1 (CD4 count <200 cells/μL)			Stratum 2 (CD4 count ≥200 cells/μL)				
	Week 0	Week 8	Week 16	Week 0	Week 8	Week 16	Week 0	Week 8
Baseline anti-H1 Ab-negative	n = 43			n = 139			n = 4	
Anti-H1 Ab responses	<10	26‡ (10–1280)	23‡ (10–1280)	<10	42 (10–1280)	36 (10–1280)	<10	135 (40–320)
Baseline anti-H3 Ab-negative	n = 37			n = 130			n = 4	
Anti-H3 Ab responses	<10	25‡ (10–640)	23‡ (10–1280)	<10	34 (10–1280)	32 (10–640)	<10	135 (40–320)
Baseline anti-H1 Ab-positive	n = 8			n = 67			n = 22	
Anti-H1 Ab responses	44 (20–320)	353 (40–1280)	208 (80–160)	54 (20–1280)	158 (20–1280)	143 (20–1280)	80 (20–640)	86 (20–640)
Baseline anti-H3 Ab-positive	n = 6			n = 73			n = 22	
Anti-H3 Ab responses	32 (20–80)	46 (20–160)	71 (20–640)	41 (20–1280)	105 (20–1280)	87 (10–1280)	59 (20–320)	66 (20–320)

*The data presented here are the geometric mean of anti-HA antibody titer. Range of the absolute titer is shown in parentheses.
†To analyze antibody responses to vaccination, patients with influenza infection were excluded from this analysis.
‡$P < 0.05$ compared with the respective value of stratum 2.
Ab indicates antibody. Change of the antibody titer from <10 to 40 U was considered a 4-fold rise.

anti-H3. Conversely, in those with a CD4 count >200 cells/μL, 16 of 67 patients and 19 of 73 patients showed more than a 4-fold rise.

Anti-H1 and Anti-H3 Antibody Responses in Patients With Influenza Illness Despite Vaccination

A total of 16 patients (5 with H1N1 strain, 8 with H3N2 strain, and 3 with B strain) had influenza illness among the vaccinated group during this study period. In the 5 patients with H1N1 illness, 3 were baseline anti-H1 antibody-negative and 2 had the antibody. Among the 3 baseline anti-H1 antibody-negative patients, 2 were infected before week 8 and 1 was infected after week 8. In the patient infected after week 8, no anti-H1 antibody was detected at week 8. In each of the 2 baseline anti-H1 antibody-positive patients, the titer was 20 U. Both patients were infected before week 8. In the 8 patients with H3N2 illness, 6 were baseline anti-H3 antibody-negative and 2 were positive for the antibody. In the 6 baseline anti-H3 antibody-negative patients, all were infected after week 8. Among these 6 patients, 4 were negative for anti-H3 antibody at week 8, whereas 2 had a 4-fold rise in the antibody before infection. In each of the 2 baseline anti-H3 antibody-positive patients, the titer was 20 U. Both patients were infected after week 8. Anti-H3 antibody at week 8 was increased to 40 U (a 2-fold rise) only in 1 patient. Overall, among the 9 infected patients (1 with H1N2 strain and 8 with H3N2 strain) in whom the antibody responses at week 8 could be evaluated, only 2 had a >4-fold rise of the antibody response before infection.

H1-Specific CD4 T-Cell Response Before and After Vaccination in Baseline Anti-H1 Antibody-Negative Subjects

H1-specific CD4 T-cell responses at week 8 were HIV VL dependent ($P < 0.005$) but not CD4 count dependent (Fig. 2A). Therefore, H1-specific CD4 T-cell responses were significantly increased by vaccination in HAART-treated patients ($P = 0.001$), because HIV VL was decreased by HAART (see Fig. 2B). In contrast, responses of HAI antibody titer were not different between HAART-treated and antiretroviral-naive patients (see Fig. 2C).

Comparison of Immune Responses to H1 Antigen at Week 8 Between Influenza A/H1N1–Infected and –Uninfected Patients

Five individuals were infected with influenza A/H1N1 during this season. HAI antibody titers at 8 weeks after the vaccination were not different between the infected and uninfected individuals. In contrast, H1-specific CD4 T-cell responses at week 8 were significantly low in the infected persons compared with those in the uninfected persons ($P < 0.05$; Fig. 3).

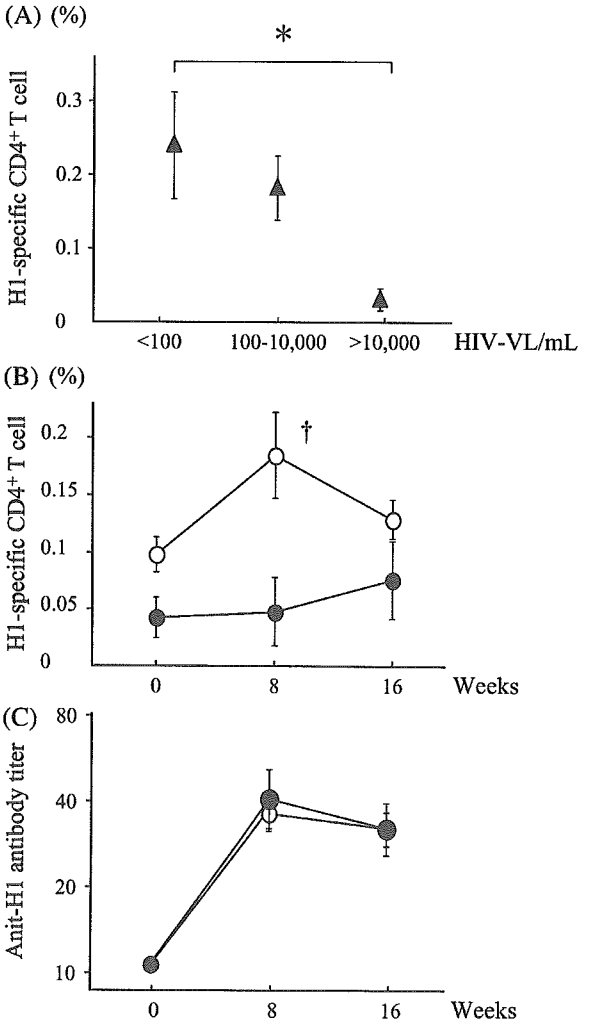

FIGURE 2. H1-specific CD4+ T-cell responses after influenza vaccine in baseline anti-H1 antibody–negative patients. A, Correlation of plasma HIV-1 viral load (HIV VL) and percentage of H1-specific CD4+ T cells. *H1-specific CD4+ T cells (▲) were significantly fewer in number in subjects with an HIV VL >10,000 copies/mL ($P < 0.005$). The number of samples with an HIV VL <100 copies/mL was 53, there were 19 samples with 100 to 10,000 copies/mL, and there were 11 samples with >10,000 copies/mL, because H1-specific CD4+ T cells were only examined in the first 10 samples per day as stated in the text. B, Changes in the percentage of H1-specific CD4+ Tcells in highly active antiretroviral therapy (HAART)–treated; (○; n = 63) and antiretroviral-naive patients (●; n = 12). †HAART-treated patients had significantly greater numbers of H1-specific CD4+ T cells at week 8 ($P < 0.01$) than antiretroviral-naive patients. C, Changes in anti-H1 antibody titer in HAART-treated (○; n = 131) and antiretroviral-naive patients (●; n = 35). Anti-H1 antibody responses were similar in both groups. Data are mean ± SEM.

DISCUSSION

Our prospective study confirmed many conclusions of previously reported small studies. First, we confirmed the protective effect of influenza vaccine in HIV-1–infected patients.[8–15] Second, anti-H1–specific and anti-H3–specific antibody responses were examined in HIV-1–infected patients after vaccination, and the responses were confirmed to be dependent on CD4 counts.[8–11]

To clarify the efficacy of a single-shot vaccination, we divided the participants by the positivity of anti-H1– and anti-H3–specific antibodies before vaccination and found that in